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# MOLECULAR CHARACTERIZATION OF THE OPAQUE-6 MUTATION OF *ZEA MAYS* L.

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Maize endosperms accumulate during development a large amount of storage proteins (zeins). The rate of zein accumulation is under the control of several regulatory loci, many of which lower the zein level, thus improving the nutritional quality of maize meals. Among these regulatory loci is *Opaque-6* (*O6*), located on the long arm of chromosome 8, which when present in recessive form determines a general reduction of zein accumulation. Curiously, the extent of growth of the *opaque-6* locus, which has been demonstrated to be allelic to the *pro-1* locus, is limited by the availability of Proline. Without Proline the mutant plants show abnormal leaves, reduced growth and lethality at the second leaf stage. Moreover, at the ultrastructural level, primary leaves of *o6* mutants exhibit an abnormal chloroplast development. Various explanations for these phenomena were conceived: 1) the mutants cannot make Proline in sufficient amounts; 2) Proline degradation is enhanced in comparison to synthesis; 3) Proline transport across intercellular compartments is blocked; 4) Proline is having some effect related to its known role as a stress reliever. More recently, it was demonstrated, by means of an *in vitro* growth assay, that the *o6* mutant can be rescued not only through Proline addition, but also by supplying Arginine, Asparagine, Glycine, Leucine, Methionine, and Tryptophan during plant growth. These findings dismantle the previously proposed explanations of *O6* functionality.

In order to increase our knowledge of the *Opaque-6* locus, we performed microarray experiments on homozygous wild type (WT) and o6 plantlets. For this purpose, mature WT and mutant seeds were sterilized and germinated in Petri dishes for 48h at 27°C. Embryos were then dissected and placed in test tubes containing basal medium, or basal medium with Proline. On the basal growth medium, the WT seedlings showed a normal phenotype, whereas the mutant seedlings exhibited the described abnormalities of leaves, reduced growth and lethality at the second leaf stage. Mutant embryos cultivated on basal medium added with Proline showed a complete recovery of the normal phenotype. Plantlets were collected after 1- and 5 days of *in vitro* growth. Subsequently, total RNA was extracted from each of the eight sample types (WT and mutant genotype; basal and Proline supplied medium; 1 and 5 days of growth). Sample extractions were performed in threefold and used to prepare Cy-3 and Cy-5 labelled hybridization probes. A 55,000 oligonucleotide maize microarray was then hybridized with the molecular probes, using a dye-swapping strategy. The collected hybridization data were first normalized at the slide level, in order to remove dye-related signal differences, and subsequently across slides. All statistical procedures were performed with the use of the lemma package for the R software suite. The obtained results will be discussed in relation with the morphological abnormalities exhibited by the homozygous *o6* mutant plants.